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Peritoneal permeability in the rat: Modulation by microfilament-active agents

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Peritoneal permeability in the rat: Modulation by microfilament-active agents. A model of peritoneal dialysis in the rat was used to determine the effects of cytochalasins on ultrastructure and peritoneal permeability to molecules of varying molecular weight. The permeability to urea, inulin, and plasma albumin were determined after intraperitoneal administration of cytochalasin B (2 to 10 $\times$ 10^{-6} M) and cytochalasins D and E (2 $\times$ 10^{-6} M). Cytochalasin B (20 $\times$ 10^{-6} M) increased the permeability to inulin, urea, and albumin by 30, 60, and 150%, respectively. These effects were, to a large degree, reversible. Cytochalasins D and E produced greater increments in permeability for all molecules; this increase was only partially reversible. Ultrastructure analysis by scanning electron microscopy revealed extensive development of membrane protuberances (zeiotic knobs) on mesothelial cells exposed to cytochalasin B. A return to a normal apical cell surface was apparent although incomplete at 24 hr. Tight junctions were not grossly altered and major changes in intramembranous junctional strands were not observed. The major effect of cytochalasins on the cell surface may be responsible for the increased permeability to urea, predominately a transcellular probe. Inulin, which follows a paracellular route, was less affected. Altered protein permeability may be due to the action of cytochalasin on the exposed capillary endothelium in subdiaphragmatic areas where the mesothelium is discontinuous.

Perméabilité péritonéale chez le rat: modulation par des agents actifs sur les microfilaments. Un modèle de dialyse péritonéale chez le rat a été utilisé pour déterminer les effets de cytochalasines sur l'ultrastructure et la perméabilité péritonéale à des molécules de divers poids moléculaires. La perméabilité à l'urée, à l'inuline, et à l'albumine plasmatique a été déterminée après administration intrapéritonéale de cytochalasine B (2 à 20 $\times$ 10^{-6} M) et cytochalasines D et E (2 $\times$ 10^{-6} M). La cytochalasine B (20 $\times$ 10^{-6} M) a augmenté la perméabilité à l'inuline, l'urée, et l'albumine de 30, 60, et 150%, respectivement. Ces effets étaient, pour une large part, réversibles. Les cytochalasines D et E ont entrainé des élévations supérieures de perméabilité à toutes les molécules; cette augmentation était seulement partiellement réversible. Une analyse de l'ultrastructure par microscope électronique à balayage a révélé le développement extensif de protubérances membranaires (boutons zéiotiques) sur les cellules mésothéliales exposées à la cytochalasine B. Un retour à une surface cellulaire apicale normale était apparent, mais incomplet, à 24 hr. Les jonctions serrées n'étaient pas très altérées, et on n'observait pas de changement important des zones jonctionnelles intramembranaires. Cet important effet des cytochalasines sur la surface cellulaire pourrait être responsable de l'augmentation de perméabilité à l'urée, un marqueur à prédominance transcellulaire. L'inuline, qui suit une voie paracellulaire, était moins affectée. L'altération de la perméabilité aux protéines pourrait être due à l'action de la cytochalasine sur l'endothélium capillaire exposé dans les zones sous-diaphragmatiques, où le mésothélium est discontinu.

The success of continuous ambulatory peritoneal dialysis (CAPD) in maintaining the well being of patients with chronic renal failure has increased the significance of understanding how molecules permeate through the peritoneal membrane. The peritoneal membrane is a complex barrier, and the various pathways for permeation of molecules during dialysis remain poorly defined [1]. Augmentation of peritoneal clearances by use of pharmacologic agents or other maneuvers has become an important objective.

Attempts to enhance peritoneal permeability to *small* and *middle* size molecules in peritoneal dialysis have so far focused on the incorporation of vasodilator compounds into the dialysate. These agents may increase peritoneal blood flow and the effective area for diffusion or convective flow by recruitment of collapsed capillaries [2–4].

There is increasing interest in the role of the cytoskeleton in epithelial cells in the control of net transepithelial transport [5]. Evidence especially supports the involvement of the submembranous microfilament system in regulation of paracellular transport through the tight junction [6]. It is known that cytochalasins B and D greatly increase the permeability of capillary walls, permitting widespread exudation of plasma albumin and globulins into interstitial spaces when administered intravenously to experimental animals [7–9]. Cytochalasins are also known to disrupt microfilaments by binding to the growing ends of polymerizing actin filaments and thereby preventing augmentation of actin monomer strands [10–12]. We therefore decided to investigate the effect of cytochalasins on peritoneal permeability to gain a better understanding of the function and structure of the transperitoneal pathways utilized by solutes of varying molecular weight.

Methods

Peritoneal dialysis was performed in 11 male Wistar rats weighing 250 to 400 g. The dialysis procedures were modifications of those described by Brown et al [3] and Alavi et al [4].

Dialysis technique. Animals were anesthetized by an intraperitoneal injection (100 mg/kg of body wt) of Inactin (BYK Gulden Konstanz, West Germany) and placed on a heated animal table. A tracheostomy was performed. Rectal tempera-

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ture was monitored throughout the experiment. To maintain constant blood levels of ^{3}H-inulin and urea, a half-normal saline solution containing 4 μCi/ml of ^{3}H-inulin with 0.5 mg/ml of carrier inulin and 50 mg/ml of urea were infused at a rate of 0.8 ml/hr into the jugular vein (Razel Pump, Stamford, Connecticut, USA). This solution (300 μl) was given to the rat as a priming dose immediately before starting the infusion.

Peritoneal dialysis was performed via a shortened, sterile catheter (McGaw Laboratories, Irvine, California, USA) inserted in the peritoneal cavity through a midline incision. To exclude net water and solute movement as a result of osmotic gradients created across the peritoneal membrane, an isotonic Ringer's lactate solution was used as the dialysate. Thirty minutes following the injection of the priming dose and infusion, several washes of the peritoneal cavity were performed with the Ringer's solution to remove accumulated ^{3}H-inulin and urea and to ensure satisfactory drainage. The latter was indicated by a full return ($\pm$1 ml) of the infused dialysate volume within 3 min.

Three control cycles were then performed using either 25 or 30 ml of warm (37°C) dialysate, depending on the size of the animal. Dialysate was infused within 30 sec, remained in the peritoneal cavity 12 min, and was allowed to drain for 3 min. Thus, the duration of each cycle was considered to be 15 min.

In the next three cycles, cytochalasins B, D, or E (Aldrich Chemicals, Milwaukee, Wisconsin, USA) were added to the dialysate so that final concentrations ranged from 1 to 20 μM. Dimethyl sulfoxide (DMSO) was used as a solvent for cytochalasins at a final concentration of 0.1%. Dimethyl sulfoxide alone (0.1%) did not affect permeability. Finally following two washes, three recovery cycles were performed using Ringer's solution alone.

Analytical techniques. In the middle of each 15-min cycle a 50-μl blood sample was obtained from the tail vein in heparinized capillary tubes for the determination of ^{3}H-inulin and urea concentration. Plasma (20 μl) was pipetted into an Aquasol solution (New England Nuclear Corp., Boston, Massachusetts, USA) and counted by a liquid scintillation counter (Mark IV-Nuclear Chicago, Chicago, Illinois, USA). The plasma urea concentration was determined from 10-μl aliquots by the method of Berthelot and Nessler [13], (Biodynamics Kit, Boehringer Mannheim Biochemicals, Indianapolis, Indiana, USA). ^{3}H-inulin and urea were measured from 200-μl aliquots of the peritoneal drainage obtained at the end of each cycle.

A micro-continuous gradient gel electrophoresis procedure was used for the separation and quantitation of proteins in 1-μl aliquots of the dialysate at the end of each cycle. The continuous gradient gels are contained in 5-μl microcaps. The analysis is identical to that previously described for the analysis of urine [14]. In selected experiments the same method was used for the analysis of plasma (diluted 1:51). Results are presented as concentrations of total protein, albumin, high molecular weight (HMW = MW > albumin) and low molecular weight (LMW = MW < albumin) fractions.

Systolic blood pressure was monitored during the course of some experiments by the indirect tail-cuff method using a pneumatic pulse transducer (Narco Biosystems), amplified and recorded with a polygraph (Grass, model 79). This method gives results comparable to direct intraarterial pressures [14].

Determination of peritoneal permeability. Because molecular transfer across the peritoneum is thought to involve only passive transfer processes, we have analyzed our data in a fashion similar to that presented by Gosselin and Berndt [15] assuming solutes move across the peritoneum exclusively by diffusion. A more extensive development of the equations was presented in a previous publication [4].

The quantity of ^{3}H-inulin, urea or albumin transferred into the peritoneal cavity per unit of time is given by a C_dV/t. The following equations therefore describe the net flux of solutes across the peritoneal membrane.

$$\frac{C_dV}{t} = P \cdot A \cdot \Delta C \quad (1)$$

$$\Delta C = C_p - C_d \quad (2)$$

In the above equations, P equals the permeability of peritoneal membrane, cm/min; A, the effective peritoneal surface area available for diffusion, an unknown parameter, cm^2; ΔC, concentration gradient for urea, ^{3}H-inulin or albumin; C_d and C_p, concentration of urea, ^{3}H-inulin or albumin in dialysate or plasma, respectively (mg/ml or cpm/ml); V, volume of dialysate (25 or 30 ml); and t, time (15 min).

This equation can be integrated to yield a term defining net flux across the peritoneal membrane during each 15-min clearance period.

$$\frac{C_dV}{t} = K[C_p - (C_d/2)] \quad (3)$$

It can be seen that K, which equals P · A, is in units of ml/min and is in effect an expression for "clearance" in which backflux of inulin, urea, and albumin from peritoneal cavity to blood is considered. Application of Eq. *(3)* yielded more reproducible and linear values for K from cycle to cycle than did the classical expression for clearance, $(VC_d/t)/C_p$, suggesting that backflux may not be negligible during a 15-min cycle. Each clearance period was 15 min, and at the end of the period, fluid in the peritoneal cavity was removed and replaced by fresh dialysate. Since there was a linear influx and a small backflux during the short time frame of each clearance cycle, the product of $C_p - C_d/2$ (the average concentration gradient) times K, should yield the rate at which probe molecules accumulate in the peritoneal cavity, C_dV/t.

Small increases in permeability to inulin (7%) and urea (16%) occur after seven to nine consecutive cycles [4]. Where appropriate, these time-dependent changes were used to correct for clearances measured in these late cycles. One to eight experiments were performed for each concentration of intraperitoneal cytochalasins tested. The permeability derived was expressed as the ratio of that measured in the same animal during control cycles. Recovery was handled in a similar manner. All results are presented as means $\pm$ SEM. The Student's *t* test for paired observations was used to test for significance.

Morphologic studies. The morphologic changes in omentum induced by various concentrations of intraperitoneal cytochalasin were evaluated by thin-section electron microscopy (EM), freeze fracture, and scanning electron microscopy (SEM). These tissues were compared to those sampled from control and recovery phases. Fixation was achieved by intraperitoneal instillation of 2 ml of a 25% glutaraldehyde solution into the

Table 1. Effect of cytochalasins on peritoneal permeability

Cytochalasins	*N*	Concentration $\times 10^{-6}$ M	Inulin			Urea		
			K_C, *ml/min*	K_E/K_C	K_R/K_C	K_C, *ml/min*	K_E/K_C	K_R/K_C
CB	3	2 to 12	0.28 ± 0.04	1.28 ± 0.04	1.07 ± 0.05	0.63 ± 0.08	1.52 ± 0.20	1.01 ± 0.09
CB	6	20	0.25 ± 0.02	1.29 ± 0.12	1.06 ± 0.16	0.68 ± 0.08	1.61 ± 0.15	1.23 ± 0.12
CD	2	2	0.22 ± 0.03	1.57 ± 0.05	1.38 ± 0.15	0.57 ± 0.23	1.64 ± 0.37	1.53 ± 0.27
CE	2	2	0.15 ± 0.03	1.59 ± 0.00	1.77 ± 0.02	0.45 ± 0.02	1.29 ± 0.04	1.62 ± 0.31

Abbreviations: K_E, permeability using test substance; K_C, permeability measured during control periods; K_R, permeability measured during recovery.

dialysate present in the peritoneal cavity at the end of the last cycle. For freeze fracture, glutaraldehyde-fixed tissue was passed through graded concentrations of glycerol up to 30% and then frozen in Freon 22 and liquid nitrogen. Tissue was fractured at −100°C and shadowed with platinum and carbon in a Denton FE-3 apparatus. For scanning EM, glutaraldehyde-fixed tissue was critical-point dried in carbon dioxide (Sorvall dryer), sputter-coated with gold at a 45° angle (Kinney SC-3 vacuum evaporator) and examined in a Jeol SEM (35CF). For thin section transmission EM, tissue was postfixed in 1% OsO_4, stained in block with a 0.5% uranyl acetate (pH = 5.1), dehydrated in graded concentrations of ethanol, and embedded in Epon. Freeze fracture and thin sections were examined in a Siemens IA electronmicroscope. Tissues obtained at control and recovery cycles were handled similarly. Morphological studies were confined in omentum.

Results

Effects on urea and inulin permeability. The effect of cytochalasins on peritoneal permeability to small molecules (urea) and medium size molecules (inulin) is presented in Table 1. Since the baseline (control) permeability varies from animal to animal, permeability changes induced by cytochalasin are expressed as the ratio of the experimental (K_E) or recovery (K_R) permeability divided by the control value (K_C). Cytochalasin B (CB) increased permeability to inulin 28% ($P < 0.05$). Greater increases (>50%) were observed with cytochalasins D and E (CD and CE). These changes were reversible at all concentrations of CB, but reversibility was less with CD and CE. Changes in permeability to urea were more pronounced (CB increased permeability to urea 52%, $P < 0.05$); reversibility followed the same pattern as for inulin. There was no significant change from control values in dialysate volume during experimental and recovery cycles with CB, CD, or CE. Absolute values for permeability revealed that the peritoneum is about 2.5 times more permeable to urea than to inulin.

Effects on protein permeability. Peritoneal permeability to protein did not increase significantly due to the dialysis procedure using Ringers lactate dialysate. Dimethyl sulfoxide at a concentration of 0.1% was included in the dialysis fluid. The average protein content for the control periods in all experiments was comparable to that presented in Table 1 (total protein = 21.5 ± 2.0 mg/dl; albumin = 13.1 ± 1.0 mg/dl; composition: HMW = 33 ± 2%, albumin = 73 ± 2%, LMW = 4 ± 0.5%). A representative experiment is presented in Table 2. Twelve consecutive 15-min dialysis cycles were performed and the concentrations of total protein and albumin and the fractional composition of protein were measured. There was no significant change in peritoneal permeability to these proteins with time (180 min).

Table 2. Effect of dialysis and time on protein content of dialysate[a]

Period[b]	Total protein *mg/dl*	Albumin *mg/dl*	HMW	Albumin	LMW
			%		
1	24.2 ± 2.8	15.9 ± 1.6	28 ± 4	69 ± 6	3 ± 1
2	28.5 ± 0.6	18.8 ± 0.6	28 ± 3	69 ± 3	2 ± 0.4
3	25.3 ± 0.6	16.7 ± 0.4	29 ± 1	69 ± 0.4	2 ± 1
4	23.8 ± 2.1	16.1 ± 1.0	25 ± 2	71 ± 4	4 ± 3

Abbreviations: HMW, high molecular weight; LMW, low molecular weight.

[a] No significant differences are seen.

[b] Each period represents the average ± SE of three 15-min cycles.

Cytochalasin B was found to increase the concentration of albumin in the dialysate in a dose-dependent fashion (Fig. 1). The peak albumin concentration was plotted on the vertical axis as percentage increment over the mean control concentration. These effects were reversible as shown in Figure 2. Note that the CB-induced protein leakage into the peritoneal cavity is non-selective with respect to molecular weight.

In experiments where plasma protein was measured we calculated a permeability to albumin ($K_{albumin}$) (Table 3). Permeability changes are again presented as the ratio of maximum permeability measured during the experimental period or the minimum value measured during the recovery cycle relative to that averaged in control cycles. The ratio of dialysate to plasma albumin concentration (C_d/C_p) is analogous to the sieving coefficient across the glomerular membranes. The control value of 53×10^{-4} is fifteen times greater than that measured in the normal rat kidney [16]. This leak of protein across the peritoneal membranes leads to significant protein loss during the entire procedure. The average total protein loss during cytochalasin B (20×10^{-6} M) experiments was equal to 12% of the calculated plasma protein pool [product of plasma volume [17] and plasma protein concentration (Table 3)]. The rate of protein loss (mg/24 hr × 100 g of body wt) was comparable to that seen in nephrotic animals (200 to 400 mg/24 hr) and far in excess of the normal protein excretion rate by the kidney (5 mg/24 hr × 100 g of body wt) [16].

Table 3 also includes values for plasma protein and albumin concentration, hematocrit, and blood pressure. These measurements show only moderate changes during the nine dialysis cycles and therefore attest to the physiological stability of the animal. The reversal of the permeability ratio to 1.3 is consist

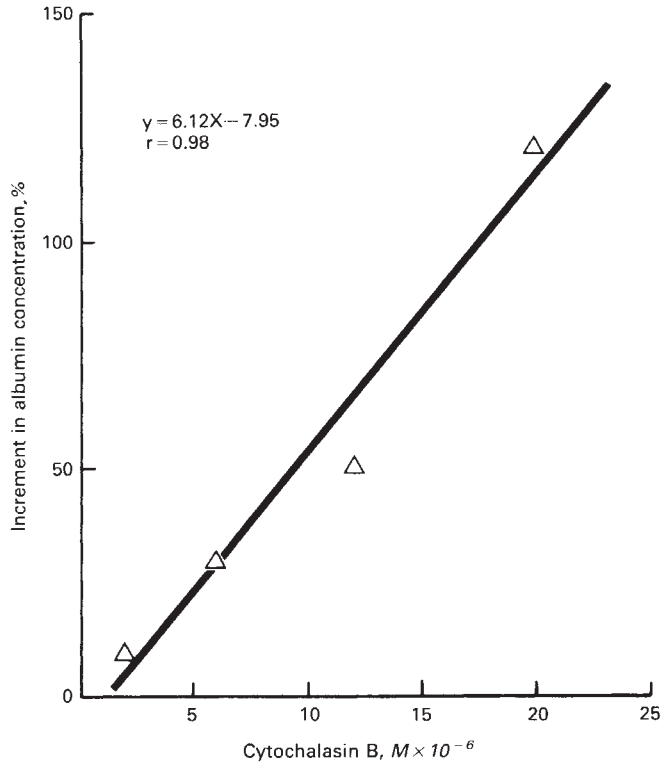

Fig. 1. *Effect of varying concentrations of cytochalasin B on dialysate albumin concentration.*

ent with the drop in dialysate protein concentration during the recovery cycles (Fig. 2).

Although not shown in Table 3, both cytochalasins E and D caused greater changes in protein permeability than cytochalasin B and their effects were not completely reversible during the time course of the recovery period. CD, at a concentration of 2×10^{-6} M, induced a sixfold increase in total protein and albumin compared to the control value. In addition, red blood cells were observed in the dialysate after administration of cytochalasin D.

The fractional contribution of albumin and HMW and LMW proteins to dialysate total protein is presented in Figure 3. The control dialysate pattern is contrasted with that seen during the peak response to cytochalasin. The fractional composition of plasma protein samples during the peak response cycle is also included. Albumin accounted for over 60% of total protein in the dialysate in control periods. Dialysate composition under both conditions was significantly different from that of plasma ($P < 0.005$). However, cytochalasins E and D abolished this selectivity and the protein pattern resembled more closely that of plasma. This was consistent with the more potent effect of these cytochalasins noted above. Administration of cytochalasin B did not change fractional composition significantly.

Electrophoresis scans of dialysis fluid and diluted plasma (1:51) from a representative experiment after the administration of cytochalasin B (20×10^{-6} M) are presented in Figure 4. Cytochalasin B increases protein movement into the peritoneal cavity quantitatively without altering relative composition, suggesting that more pores are opened rather than existing pores are enlarged.

Morphological changes induced by cytochalasins. By thin section EM (Fig. 5) structural changes in the mesothelial cell were quite subtle and consisted of some thickening and clumping of microfilaments in submembranous regions and adjacent to tight junctions. However, in focal regions, zeiotic evagination of the apical membrane was observed and in these regions, submembranous microfilaments were fragmented and their orientation within microvilli and in submembranous regions was obviously disrupted. In most microvilli integrity of the microfilamentous core was largely preserved. On scanning electronmicrographs, it is apparent that this increase in microvillous growth is very extensive (Fig. 6 **A** and **B**). Protrusions of the apical surface appear more as a web rather than as true microvilli. When exposed to cytochalasin B (20×10^{-6} M), these apical membrane protuberances become much more prominent, both with respect to their length and thickness. These membrane protuberances herniate from the apical cell surface and are probably identical to a "zeiotic knob" noted in thin section electronmicrographs (Fig. 5).

When cytochalasin B was removed and peritoneal membranes were allowed to recover (30 min, 2 and 24 hr), a return to normal configuration of the mesothelial cell apical surface was apparent although incomplete (Fig. 7 **A** and **B**). On scanning electron microscopy, the effect of cytochalasins D and E on formation of zeiotic knobs was more pronounced than with cytochalasin B.

Tight junctions did not appear disrupted by cytochalasin B exposure (Fig. 5). Even in freeze fracture replicas, no major differences were seen in the tight junctional intramembranous structure of mesothelial cells when control was compared to cytochalasin B (Fig. 8 **A** compared to **B**). Irregularities in the strand meshwork were seen more frequently after cytochalasin B exposure, but this could reflect differences in tissue processing [18]. However, the frequency of an expansive fracture through mesothelial cell membranes was so low that a morphometric analysis of tight junctional structure was impractical.

Capillaries within the fabric of the omentum did not reveal gross abnormalities either in thin section or freeze fracture electronmicrographs. The most obvious changes as described above were in the apical membrane of mesothelial cells.

Discussion

Recently, evidence supports the participation of cytoskeletal elements in the control of paracellular permeability in epithelial membranes [5, 6]. The data presented in this paper represent the first attempt to alter permeability in peritoneal dialysis by use of agents that disrupt the microfilament system. The evidence that cytochalasins augment peritoneal permeabilities confirm and extend the previous observations of Glinsukon et al [7], Hayakawa et al [8], and Glinsukon, Shank, and Wogan [9]. Investigating the toxicology of cytochalasins D and E, these investigators reported that animals receiving a lethal dose intravenously developed rapid exudation of plasma proteins into pleural and peritoneal cavities, and death from shock within 2 to 19 hr. These investigators further concluded that the effects were most likely due to the drug acting directly on the capillary walls. The lethal doses at which these effects were observed was 10 mg/kg with cytochalasin D for Swiss mice and

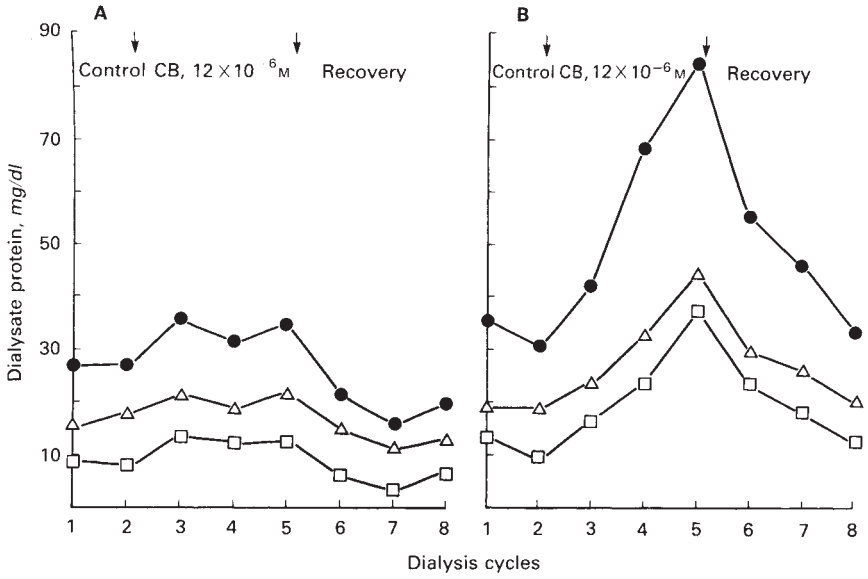

Fig. 2. *Changes in dialysate protein concentration during administration of cytochalasin B.* Symbols are: ●, total protein; △, albumin; □, high molecular weight.

Table 3. Effect of cytochalasin B on albumin permeability, plasma composition, and blood pressure

	Control	Cytochalasin B	Recovery
$K_{albumin}$, *ml/min*	0.008 ± 0.001	0.020 ± 0.002	0.010 ± 0.001
$K/K_{control}$	1.0	2.5 ± 0.2	1.3 ± 0.1
$(C_d/C_p)_{albumin}$, $\times 10^4$	53 ± 5	114 ± 11	62 ± 4
Plasma protein, *g/dl*	6.3 ± 0.3	6.1 ± 0.4	5.7 ± 0.3
Plasma albumin, *g/dl*	2.8 ± 0.1	2.7 ± 0.1	2.5 ± 0.1
Hematocrit, %	45 ± 1	46 ± 1	47 ± 2
Systolic blood pressure, *mm Hg*	119 ± 9	103 ± 6	95 ± 9

[a] Values are means ± SE; the number of animals is five. Cytochalasin B was added to the dialysate at a concentration of 20 × 10^{-6} M.

2.6 mg/kg and 4.6 mg/kg for mice and rats, respectively, with cytochalasin E [7–9].

Cytochalasins are fungal products that alter cell morphology. Cytochalasin B binds to the growing end of filamentous actin, causes depolymerization [19], and changes in microfilament assembly which lead to alteration in motility-related processes such as locomotion and cytokinesis [11, 12, 19]. Zeiosis, in which there is focal budding of the cell membrane, is presumably due to loss of cytoskeletal anchoring of the cell membrane. Another biologic effect of cytochalasin B is alteration of transport across the plasma membrane. Cytochalasin B is one of the most potent competitive inhibitors of hexose and nucleoside transport [10, 20, 21]. The effect is mediated by cytochalasin B binding to some part of the glucose transport carrier [22]. Cytochalasins D and E do not bind to the glucose carrier protein and therefore do not have the transport inhibitory effect [10, 22]. Exactly which of these biological actions is responsible for the increase in permeability of the peritoneum is uncertain. In this study cytochalasins were exposed to the outside surface of the peritoneum. Since cytochalasins may have a limited permeability across cell membranes, access to the capillary endothelium may be hindered and perhaps limited to capillaries not covered by mesothelial cells. The roles played by the various membranes that constitute the peritoneum in the control of diffusion of molecules of different size, charge, and shape remain poorly understood. In the omentum, a single layer of mesothelial cells rests on a collagenous matrix containing capillaries, lymphatics, elastic fibers, histocytes, and fibrocytes [24–26]. Omentum not encircling the gut, consists of two diametrically apposed mesothelial cell layers in a sandwich type array on each side of the matrix. Microvilli-like projections 0.1 μm in length are present on the apical surface of the mesothelial cells. These microvilli and the adjacent cytoplasmic regions have a cytoskeletal architecture not too dissimilar to that in resorptive epithelia [27]. In addition, in the subdiaphragmatic areas, mesothelium is discontinuous, leading to the formation of "lacunae" or pores 4 to 12 μm in diameter [28]. In these regions capillaries would presumably be in intimate contact with the peritoneal cavity.

Several mechanisms exist to explain solute transport across the peritoneum: diffusion and ultrafiltration dependent on concentration and pressure (osmotic and hydrostatic) gradients, and pinocytotic transport through mesothelial and endothelial cells [26]. Which membranes are rate-limiting barriers for solute transport remains unclear. The barriers include: *(1)* the capillary wall and its basement membrane, *(2)* the unstirred interstitial fluid, and *(3)* the mesothelium. The mesothelium contains both transcellular and paracellular pathways. For small water-soluble molecules such as urea, transcellular diffusion across the capillary and mesothelial cells plus the interstitium is undoubtedly the most important pathway, particularly when a relative effective area for diffusion (transcellular vs. paracellular) is considered. For larger or ionized molecules, evidence supports the paracellular pathway as being the major final path for transport [29]. For example, passage of horseradish peroxidase (molecular weight, 40,000 daltons) through tight junctions of rat mesothelium has been demonstrated [30]. Nevertheless, even though tight junctions of mesothelial cells may be

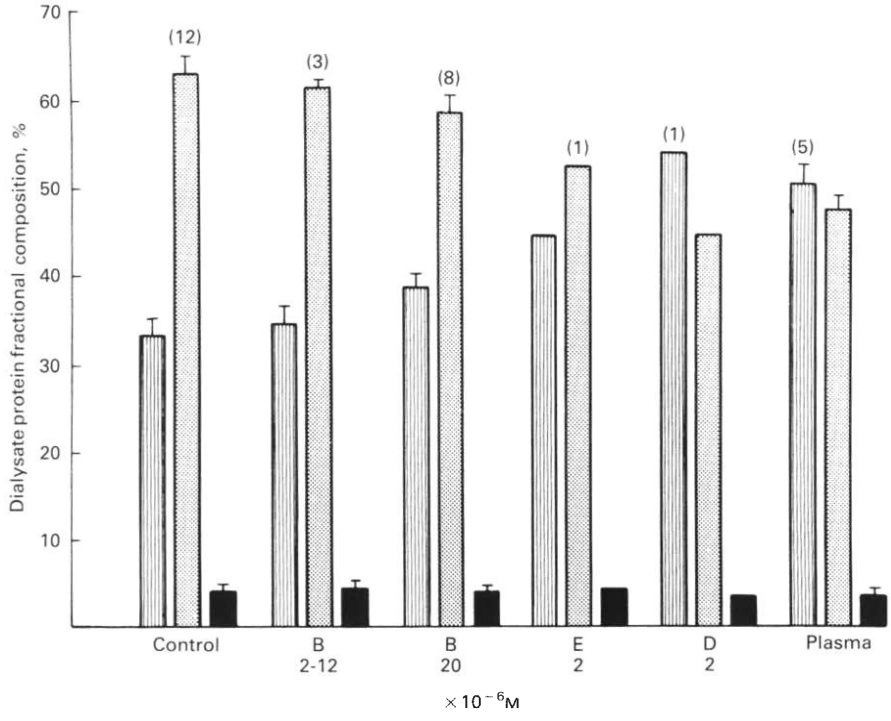

Fig. 3. *Fractional protein composition of dialysate and plasma.* Numbers in *parentheses* denote the number of animals. Data are given where appropriate as the mean ± SEM. Symbols are: ◫, high molecular weight; ▨, albumin; ■, low molecular weight.

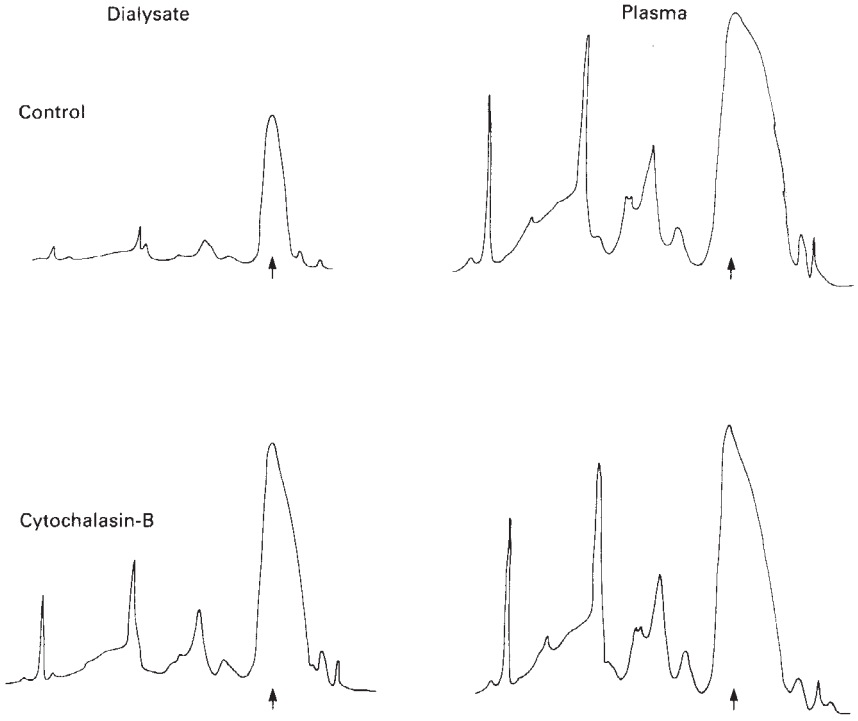

Fig. 4. *Electrophoretic analysis of plasma and dialysate.* The direction of migration is from left to right. The *arrows* indicate the albumin band in the densitometer scan. The sample volume is 1 μl.

relatively "leaky," these structures may still be the rate-limiting barrier for medium-sized molecules such as inulin [27]. For many proteins, the transcellular pinocytic pathway may also be important [26]. In addition, it is more likely that the lacunal region, where the capillary endothlium is in direct contact with peritoneal fluid, is the major site of protein flux into the peritoneal cavity.

Physiologic studies using dextrans (molecular weight, 10,000 to 90,000) have described the peritoneal membrane as a mosaic of small and large "pores" [1]. While "pores" having an effective radius of 15 to 20 Å constitute the major pore area, another population of "pores" with an effective radius of 70 Å constitutes 0.01% of the total "pore" surface area. Perhaps the subdiaphragmatic lacunae represents the 70 Å "pores" in these

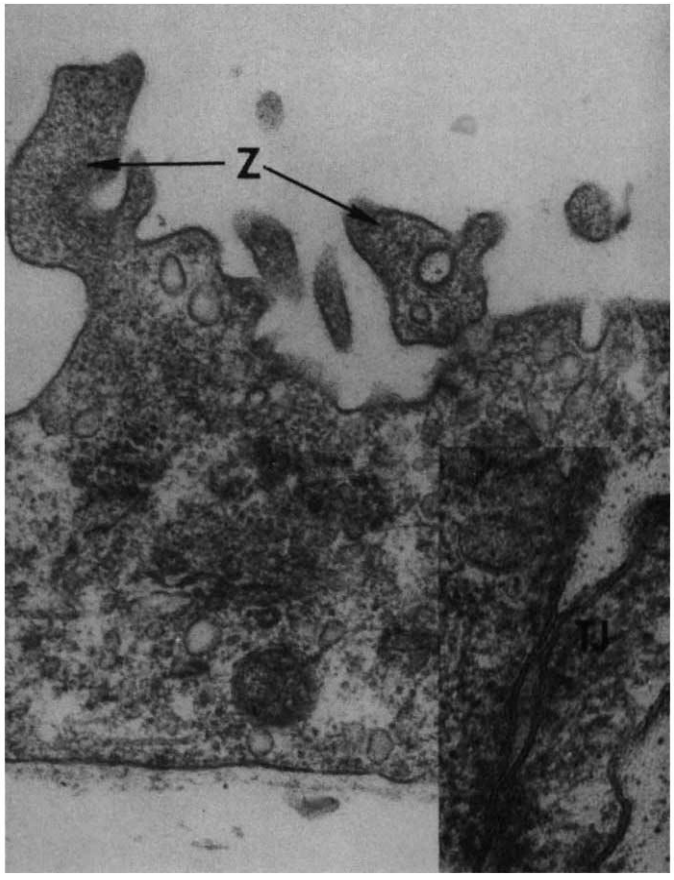

Fig. 5. *Electron micrograph of omentum after exposure to cytochalasin B.* Note zeiotic protuberances in the apical membrane of this mesothelial cell designated by the *arrows*. Microfilaments in these regions are no longer filamentous but appear as fragments. Microtubules and mitochondria appear normal. (×40,000) An intact tight junction (TJ) from the same mesothelium is shown in the *insert*. (×66,000)

studies. The fact that cytochalasin B reversibly increased permeability to protein suggests that a major effect was on the population of subdiaphragmatic capillaries. Since selectivity of protein diffusion remained unchanged with cytochalasin B (Fig. 4), it would appear that there was an increase in the number of lacunae or alternatively more capillary pores were made available for diffusion. In contrast the loss of selectivity with cytochalasins D and E and lack of recovery suggest an irreversible increase in pore diameter.

Cytochalasins when administered intravenously can indeed increase local blood flow [31, 32]. Natori [33] has measured the "irritative toxicity" of these drugs by intradermal injections at concentrations of 20 to 200 μM. Since considerable local inflammatory responses developed in the injected areas, increased peritoneal blood flow may explain some of the effect on permeability reported in this paper. This mechanism remains open to future exploration. We do not believe that alterations in blood flow can explain all the effects since differential effects on permeability were clearly evident. Peritoneal clearance is relatively insensitive to variation in mesenteric blood flow [34]. Aune [35] observed that peritoneal blood flow has to drop by 80% before any change in peritoneal clearance is observed.

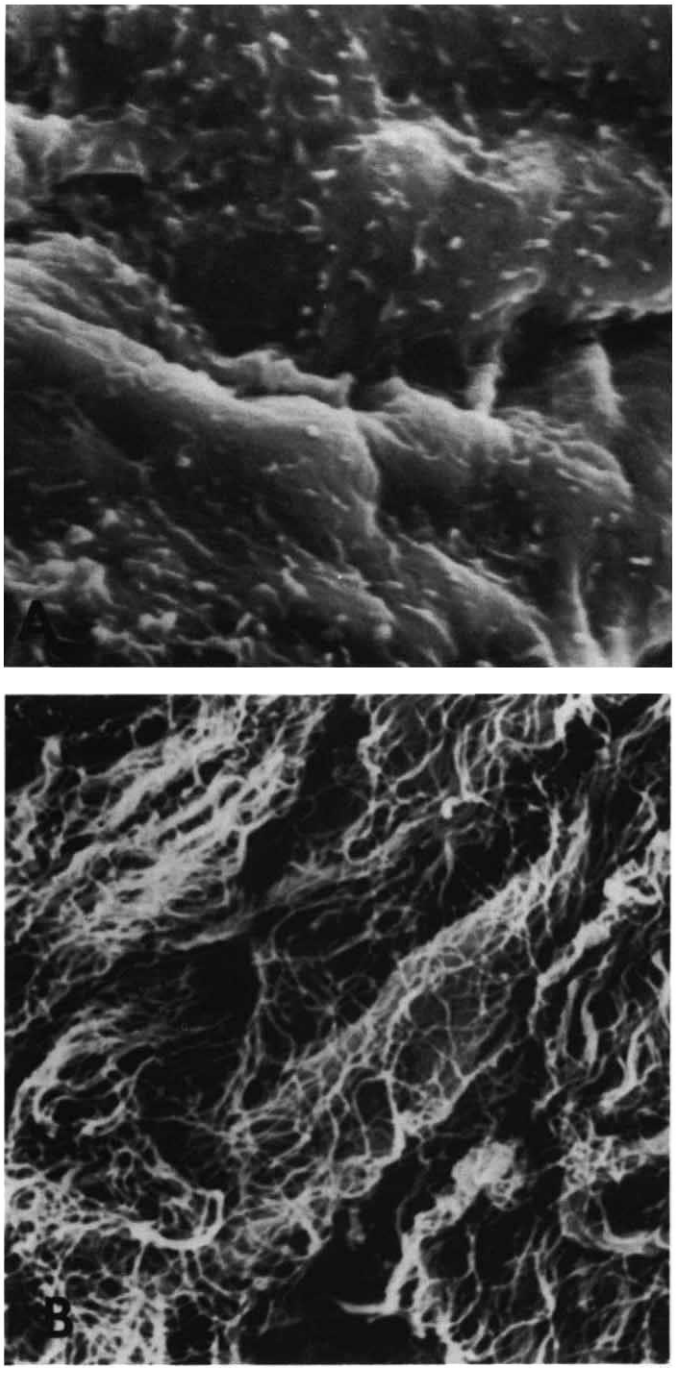

Fig. 6. *Scanning electron micrograph of omentum.* **A** *Control.* **B** *45-min exposure to 20 × 10^{-6} M cytochalasin B.* Note the extensive extrusion of cytoplasm (zeiosis). (×6000)

Zeiotic knobs form as the result of displacement and detachment of the cortical submembranous microfilaments which form a continuous barrier between the endoplasm and inner face of the plasma membrane [36]. Cytochalasin B enhances cell contraction. This allows displacement of the microfilament web and herniation of endoplasmic component of the cytoplasm follows. Cells of epithelial origin show zeiotic knobs more readily. Although omentum is mesothelial in origin, it showed marked

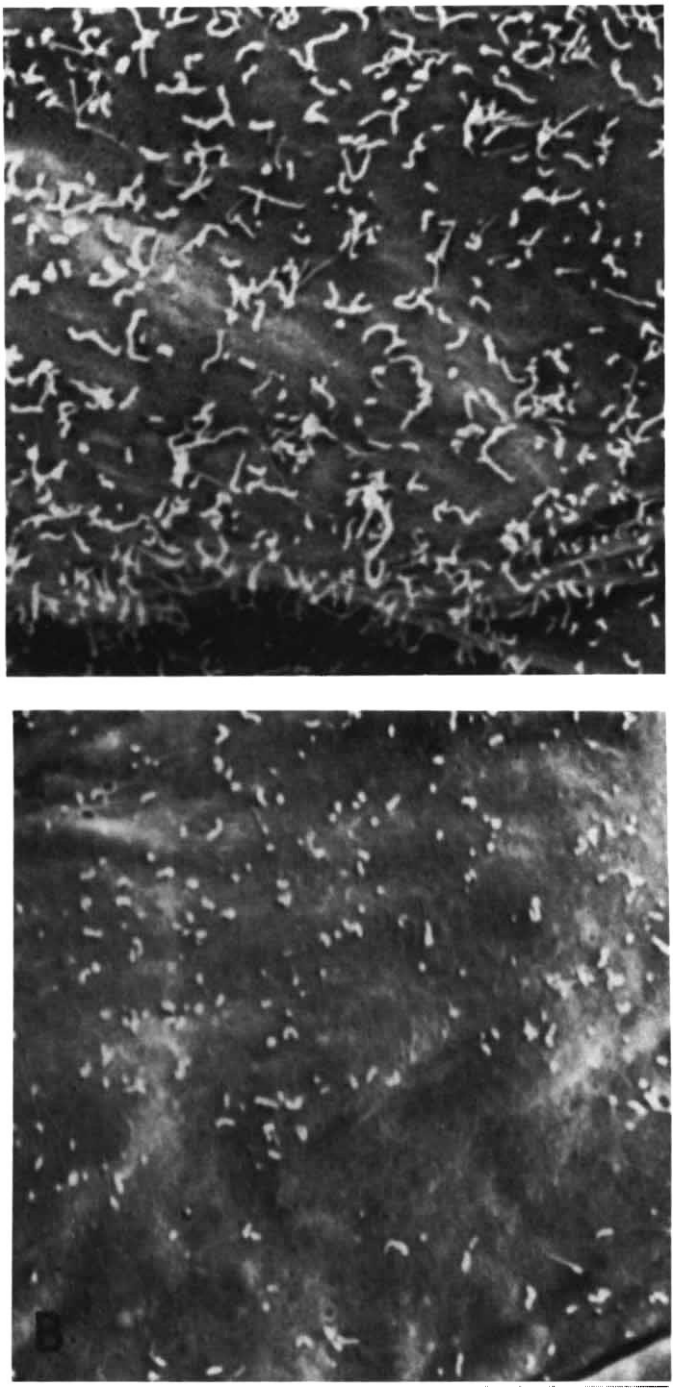

Fig. 7. *Scanning electron micrograph of omentum.* **A** *Two and one-half hours after CB removal.* Zeiotic knobs are less frequent but still present. **B** *Twenty-four hours after CB removal.* Note the return to the control condition. (×6000)

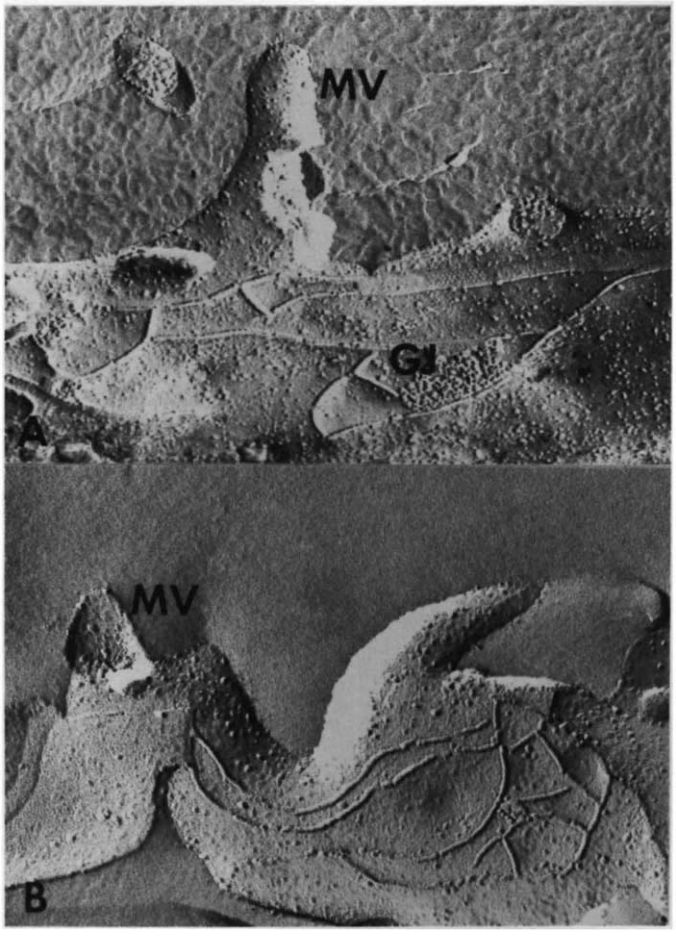

Fig. 8. *Intramembranous structure of mesothelial cell tight junctional region from the omentum as revealed by freeze fracture electronmicroscopy.* **A** *Control.* (×68,000) **B** *After exposure to cytochalasin B (20 × 10^{-6} M) dissolved in peritoneal fluid 30 min prior to fixation.* The peritoneal cavity is indicated by cellular projections labeled MV. Gap junctions (GJ) are frequently observed in tight junctional regions of the plasma membrane. (×68,000)

zeiosis when exposed to cytochalasins. Zeiotic knobs were not rapidly reversible as reported for epithelial cells.

Since a major effect of cytochalasins is on the cell surface membrane, it is likely that these drugs would alter the transcellular pathway rather than the paracellular. Since urea can pass both transcellularly and paracellularly, any possible perturbation of tight junction permeability with its small area relative to that of the mesothelial cell, is unlikely to lead to substantial changes in permeability to urea. However, permeability to inulin which probably passes only paracellularly would be more affected if there was any alteration in tight junctional permeability induced by cytochalasin B [5, 6]. Inulin permeability changed to a lesser degree than urea with cytochalasin B, which is consistent with the absence of gross alterations in tight junctions (Figs. 5 and 8).

We propose that cytochalasin B effects on intraperitoneal protein flux were predominantly due to alterations in capillary permeability. CB (20×10^{-6} M) had its greatest effect on permeability to protein: K_E/K_C for inulin was 1.29; urea, 1.61; and albumin, 2.50. A clear dose-response relationship is evident (Fig. 1).

The potential use of agents active on cytoskeletal function in reversibly altering peritoneal permeability during dialysis deserves further exploration. Such agents may serve as tools in advancing our understanding of barriers to peritoneal transport. Most importantly, we have utilized these compounds to in-

dicate that the peritoneal membrane consists of at least three rate-limiting barriers, each of which has its own size selectivity. Perhaps these barriers can be therapeutically manipulated to augment clearance of specific types of molecules. Our data show that cytochalasin B is a potent compound in augmenting peritoneal permeabilities and that its effects are partially reversible. The structure activity relationships and the therapeutic role of potentially less toxic cytochalasin derivatives in altering permeability remain an area for future exploration.

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